ACTA UNIVERSITATIS LODZIENSIS
FOLIA BIOCHIMICA ET BIOPHYSICA 5, 1986

A. Kubiak, K. Brudzyńska, Z. Walter

THE EFFECT OF ZINC IONS ON TRANSCRIPTION IN EUCARIOTIC SYSTEM

Purified DNA-dependent RNA polymerases A, B and C isolated from calf thymus contain a significant amount of zinc. Atomic absorption spectroscopy revealed the presence of 6.7, 5.35 and 2.6-4.1 g-atoms of zinc per mole of polymerase A, B and C respectively. These enzymes are inhibited by treatment with 1.10-phenantroline at concentrations varying from 10^{-5} to 10^{-4} M. However, the addition of zinc ions does not restore fully the activity of 1.10-phenantroline treated enzymes. Exogenous zinc ions reduce in vitro an overall RNA synthesis catalysed by RNA polymerases from calf thymus. In addition to the sites which bind zinc in a specific and stoichiometric way these enzymes possess other classes of binding sites with high and low affinity. Occupancy by exogenous zinc of these additional binding sites inhibits polymerase activity. Additionally, rate of RNA synthesis in isolated calf thymus nuclei, was examined in presence of 1.10-phenantroline and exogenous zinc ions. Similarly as in the case of purified polymerases, transcriptional process in nuclei was inhibited by both of these agents.

Introduction

Zinc is essential for the growth and proliferation of cells from all living organisms. Zinc is necessary for normal nucleic acid synthesis and function [1]. It is well known that template dependent DNA and RNA polymerases are metaloproteins containing zinc [2-11]. Removal of zinc from enzyme molecules by treatment with chelating agents resulted in loss of their enzymatic function [3, 7-9]. It would suggest that zinc ion is required for enzymatic activity. According to Mildvan's

model zinc is involved in the interaction between polymerase and 3'-OH terminus of a growing RNA or DNA chain [12, 13]. Therefore, the present study is an attempt to specify the role of zinc played in activities of DNA-dependent RNA polymerases from calf thymus.

Matherials and methods

Purification of RNA polymerases

Calf thymus polymerases A, B and C were isolated and purified as described previously [14]. Further purification was performed by chromatography on DNA-agarose columns prepared according to S c h a l l e r et al. [15].

Isolation of nuclei

Calf thymus nuclei were isolated and purified as described by L i f r e y and M i r s k y method [16]. Purity of the nuclear fraction was checked as a routine with the light microscope.

RNA polymerase assay

The standard incubation mixture (0.25) ml contained 80 mM Tris-HCl (pH 7.8), 4mM 2-mercaptoethanol, 0.1 mM dithiothreitol, 0.1 mM EDTA, 4 mM $MnCl_2$, 12% glycerol, 20 μg of native DNA (type V, Sigma), 1 mM CTP, 1 mM GTP, 1 mM ATP, 0.5 mM UTP, and 2 μCi (^{3}H)-UTP and 100 μl of the enzyme. The assay mixture was incubated for 10 min. at 37°C. The radioactivity in the acid insoluble reaction product was determined in a Beckman scintillation counter.

RNA synthesis in isolated nuclei

The standard reaction mixture (0.25 ml) contained: 12% glycerol, 5 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.8), 0.1 mM EDTA, 0.1 mM dithiotreitol, 0.1 M $(NH_4)_2SO_4$, 0,5 mM: ATP, GTP, CTP, 0.1 mM UTP and 4 μCi (^{3}H)-UTP. The reaction was started by addition of 100 μl of nuclei suspension (10^7 nuclei/ml). The reaction mixture was generally incubated at 25°C for 30 min. TCA precipitable counts were determined as above.

Metal analysis

Zinc content in polymerase preparations was determined at 213.86 nm using an atomic-absorption spectrometer (Unicam SP 192). All samples were mineralized by incubation at 100°C for 10 min in $H_2O_2/HClO_4$ mixture (1 : 1).

Protein determination

Enzyme concentrations were measured according to S c h a f f n e r and W e i s s m a n n [17] with bovine serum albumin as standard.

Results and discussion

The purified polymerases A, B and C used in all experiments, were DNA dependent enzymes. Polymerase A was completely insensitive to α-amanitin up to the concentration of 200 μg/ml. For polymerase C 60 percent of inhibition of enzyme activity was observed under these conditions and polymerase B was fully inactivated in the presence of 1 μg/ml of α-amanitin. The specific activity of the enzymes was 130, 270 and 340-400 units per mg protein for polymerases A, B, and C respectively.

Table 1 illustrates zinc contents of calf thymus RNA polymerases. Direct measurements by using atomic absorption spectrometry show that metal contents of the RNA polymerases A, B and C

Table 1

Zinc content of calf thymus RNA polymerases
Zawartość cynku w polimerazach RNA z grasicy cielęcej

RNA polymerase*	Protein concentration** (mg/ml)	Zinc concentration*** (μg/ml)	Zinc content (g-atoms/mole)
A	3.25	2.35	6.7
B	2.00	1.16	5.35
C	0.85-1.20	0.24-0.53	2.6-4.1

*Molecular weight of polymerases was 600 000 according to Chambon [19].

**Protein was determined as described by Scheffner and Weisemann [17].

***Zinc was measured by atomic-absorption analysis in 1 ml samples.

amounts to 6.7; 5.35; 2.6-4.1 g-atoms of zinc per mole of enzyme, respectively. Endogenous zinc is essential for enzyme activity of polymerases involved in synthetizing reaction but the role of this metal in an enzymatic activity has hot been precisely stated.

Fig. 1 illustrates effect of different concentrations of 1.10-phenantroline on RNA polymerase A, B and C activities. Similarly, as other eucariotic polymerases, the RNA polymerases isolated from calf tyhmus were inhibited by chelating agent of zinc ions, with pKi values of 4.9; 4.65 and 4.5 for A, B and C enzymes, respectively. Instantaneous inhibition of RNA polymerase activities by 1.10-phenantroline should be attributed to the interaction of the chelator with the protein bound zinc. This may occur either due to binding to the metal ion and blocking the active site or removing the endogenous metal ion. Our observation that addition of exogenous zinc to phenantroline-inhibited enzymes did not restore the full activity of polymerase B and C led us to investigate in vitro effect of zinc ions on the activity of polymerases A, B, and C not treated with this inhibitor. The exogenous zinc ions added to the reaction mixture generally reduced the overall RNA synthesis.

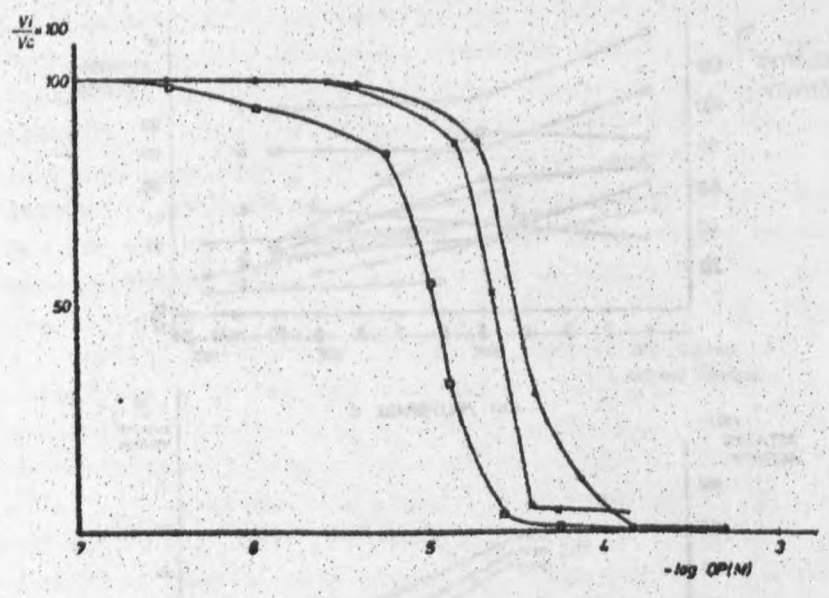
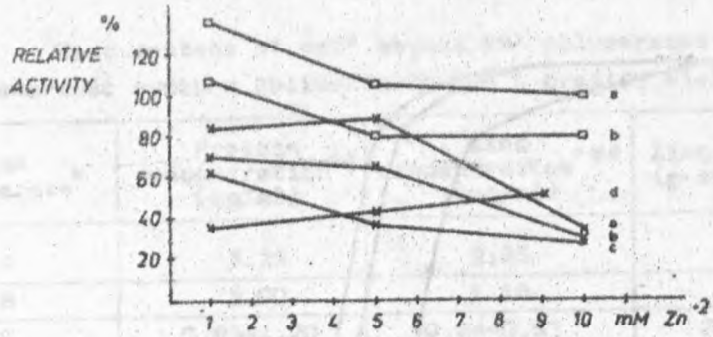


Fig. 1. Effect of 1.10-phenantroline on RNA polymerase A, B and C activities. 1.10-phenantroline was present at 10^{-6} to 10^{-3} M and enzyme protein at 10 µg per the incubation mixture. V_i is the velocity in the presence of inhibitor, V_c is the velocity in its abcence. □—□ polymerase A, x———x polymerase B, o——o polymerase C

Wpływ 1,10 phenantroliny na aktywność polimeraz RNA A, B i C. Stosowano stężenia 1,10-phenantroliny od 10^{-6} do 10^{-3} M, a białka enzymatycznego 10 µg na 250 µl mieszaniny inkubacyjnej. V_i - aktywność enzymów w obecności inhibitora, V_c aktywność kontroli. □—□ polimeraza A, x———x polimeraza B, o——o polimeraza C

Figure 2 shows inhibiting effect of exogenous zinc ions on RNA polymerase A, B and C activities. The presence of zinc in the incubation medium of polymerases A and C did not cause significant changes in their activities, especially at a low zinc concentration. A rapid decrease in the overall RNA synthesis catalysed by polymerase B was observed in the whole examined range of zinc concentration.

Effect of enzyme concentration on RNA polymerase A, B and C activities in the presence of exogenous zinc ions was examined as well. As shown in Fig. 3 the significant decrease in polymerase B activity was independent of protein concentration. In the case of polymerases A and C the magnitude of inhibiting



RNA POLYMERASE C

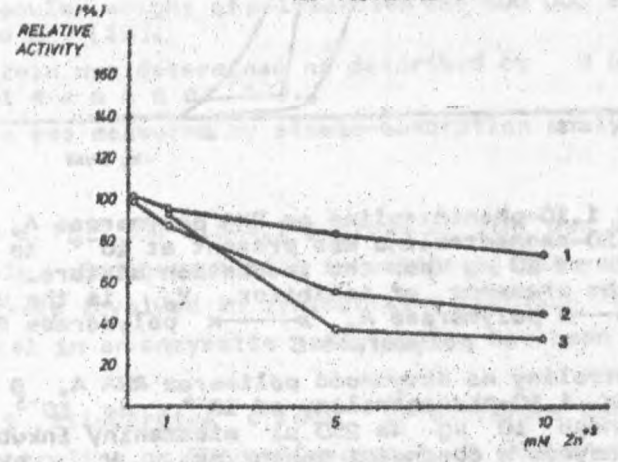


Fig. 2. Effect on exogenous zinc ions on RNA polymerase A, B and C activities. The reaction mixtures contain 1 to 10 mM Zn^{2+} and RNA polymerase samples at the following concentrations - polymerase A: a) 2.07 mg and b) 1.53 mg; polymerase B: a) 1.7 mg, b) 0.86 mg, c) 0.10 mg, d) 0.08 mg; polymerase C: 1) 12.5 μg, 2) 10.0 μg, 3) 9 μg, □—□ polymerase A, x——x polymerase B, o——o polymerase C

Wpływ egzogennych jonów cynku na aktywność polimeraz A, B i C. Mieszanina reakcyjna zawierała od 1 do 10 mM Zn^{2+} przy następujących stężeniach białka enzymatycznego - polimeraza A: a) 2,07 mg i b) 1,53 mg; polimeraza B: a) 1,7 mg, b) 0,86 mg, c) 0,1 mg, d) 0,08 mg; polimeraza C: 1) 12,5 μg, 2) 10 μg, 3) 9 μg. □—□ polimeraza A, x——x polimeraza B, o—o polimeraza C

effect of zinc is dependent on the zinc/enzyme concentration ratio. It is clearly indicated that the influence of exogenous zinc ions on polymerase A and C activities is a function of enzyme concentration. The increasing amount of enzyme protein

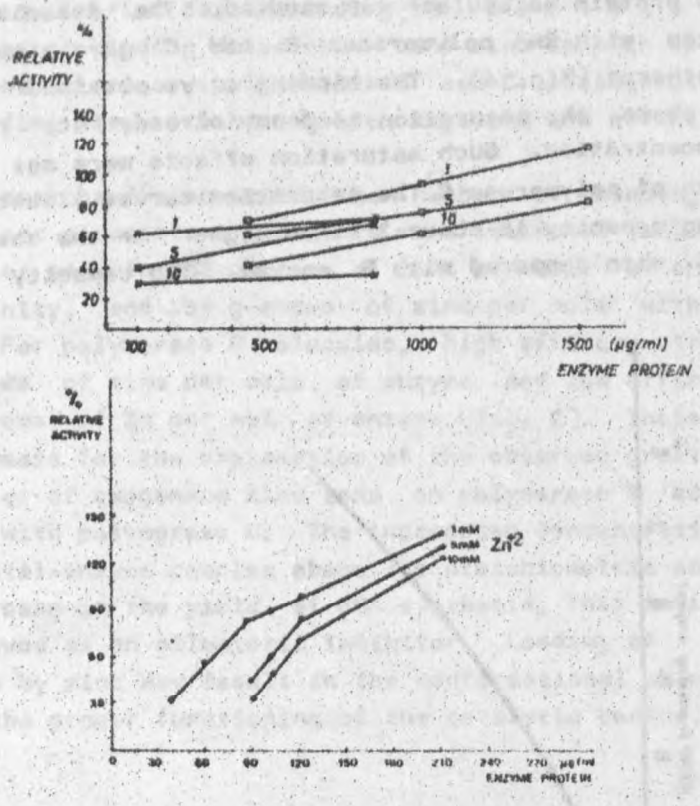


Fig. 3. Effect of enzyme concentration on RNA polymerase A, B and C activities in the presence of exogenous zinc ions. Solutions of 1.5 and 10 mM Zn^{2+} were mixed with polymerase samples containing enzyme proteins as indicated. □——□ polymerase A, ×——× polymerase B, o——o polymerase C

Wpływ stężenia enzymu na aktywności polimeraz A, B i C w obecności egzogennych jonów cynku. Roztwory 1, 5 i 10 mM Zn^{2+} były inkubowane z próbkami białek enzymów w ilościach wykazanych na wykresie. □——□ polimeraza A, ×——× polimeraza B, o——o polimeraza C

resulted in the decrease of inhibitory effect of zinc. "Dilution" of inhibitor (Zn) by enzyme protein led to reaching special enzyme/zinc concentration-ratio above which the stimulation of RNA synthesis was observed (Fig. 3). These data suggest the existance of a great number of enzyme binding sites for zinc ions. However, only a small amount of exogenous zinc ions can bind to RNA polymerase A and C without changing their original activity. To determine in detail the quantitative correla-

tion between zinc ions and polymerase activity, the binding of $^{65}Zn^{2+}$ to protein molecules was studied. The association of ^{65}Zn isotop with RNA polymerases B and C gave simple saturation isotherms (Fig. 4). The binding curve obtained for polymerase B shows the saturation tendency already at relatively low Zn concentration. Such saturation effects were not observed in the case of polymerase C. The saturation curves illustrate that the binding capacity is about 3 times higher in the case of polymerase C when compared with B enzyme. The capacity and af-

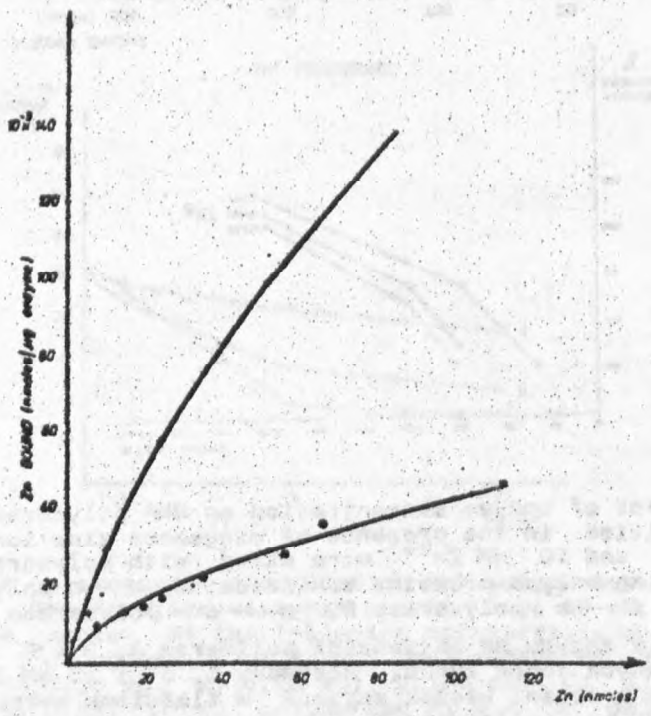


Fig. 4. Titration curves of RNA polymerases by zinc ions. $^{65}Zn^{2+}$ solutions (4 µl) of increasing concentration were mixed with 40 µl of enzyme. Following 1 h incubation at 37°C enzyme bound zinc was precipitated, collected on the Milipore filters and measured in liquid scintillation counter. o——o polymerase B, ×——× polymerase C

Krzywe wysycenia polimeraz RNA jonami cynku. Roztwory $^{65}Zn^{2+}$ (4 µl) o wzrastających stężeniach inkubowano z 40 µl białka enzymatycznego. Po 1 h inkubacji w 37°C $^{65}Zn^{2+}$ związany przez białko był strącany, osadzany na sączkach Millipore, a jego zawartość mierzona w liczniku scyntylacyjnym Beckmana. o——o polimeraza B, ×——× polimeraza C

finity for the zinc-polymerases interaction were estimated by plotting equilibrium data according to S c a t c h a r d [18]. In addition to binding sites which bind zinc ions in specific and stoichiometric manner, polymerases B and C possess other classes of binding sites exhibiting both high and low affinity to this metal.

From scatchard's equation the number of binding sites and the apparent association constants were estimated [18]. Polymerase C molecules can bind 38 g-atoms of zinc per mole of enzyme with high affinity, and 159 g-atoms of zinc per mole with low affinity. For polymerase B molecules, high affinity sites bind 9.4 g-atoms of zinc per mole of enzyme and low affinity sites 47.2 g-atoms of Zn per mol of enzyme (Tab. 2). These data may provide basis for the explanation of the observed greater inhibition effect of exogenous zinc ions on polymerase B activity as compared with polymerase C. The increasing concentration of zinc in the metal-enzyme complex above the stoichiometric amount leads to a decrease in the yield of RNA synthesis. This indicates that zinc behaves as an allosteric inhibitor. Loading of polymerase molecules by zinc may result in the conformational changes which prevent the proper functioning of the catalytic center. The dif-

T a b l e 2

A number of binding sites for exogenous zinc ions in DNA dependent RNA polymerases molecules, from calf thymus

Ilość miejsc wiążących egzogenne jony cynku w polimerazach RNA zależnej od DNA, z grasicy cielęcej

RNA polymerase	High affinity sites		Low affinity sites	
	$\frac{\text{g-atoms } Zn^{2+}}{\text{mole}}$	$\frac{K_a}{M}$	$\frac{\text{g-atoms } Zn^{2+}}{\text{mole}}$	$\frac{K_{aI}}{M}$
C	38	$13.5 \cdot 10^{-9}$	159	$98 \cdot 10^{-9}$
B	9.4	$4.8 \cdot 10^{-9}$	42.2	$97 \cdot 10^{-9}$

N o t e: Aliquots containing 1.95 µg of polymerase C and 5.08 µg of polymerase B proteins were incubated with varying amounts of $^{65}Zn^{2+}$ (0-120 nmoles) in the total volumes of 100 µl. The binding data were plotted according to S c a t c h a r d's equation [18].

ferences in the inhibitory action of zinc observed for polymerases B and C support the previous data showing that these polymerases are structurally distinct enzymes [19, 20]. Above mentioned results, obtained in vitro for RNA polymerase A, B and C from calf thymus, prompted us to investigate the effect of O-P and exogenous zinc ions on RNA synthesis in functionally active nuclei isolated from this source.

Rate of RNA synthesis in isolated calf thymus nuclei, was examined, in the presence of increasing concentrations of 1.10--phenantroline. Similarly, as in the case of purified polymera-

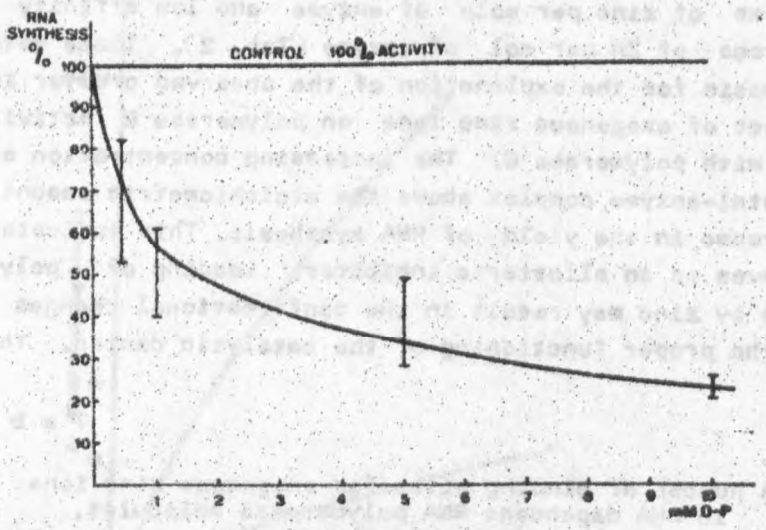


Fig. 5. Inhibiting effect of 1.10-phenantroline on RNA synthesis in isolated calf thymus nuclei. Nuclei (2 x 10^7 nuclei/ml) were incubated in presence of increasing concentrations of 1.10-phenantroline (0.5-10 mM) for 1 h at 37°C. After that 100 µl samples of nuclei suspension were added to 250 µl standard reaction mixture and incubation was performed at 25°C for 30 min. TCA precipitable counts were determined after spotting the nuclei on Whatman glass fibre filters (Control: 100% RNA-synthesis in nuclei without incubation with 1.10-phenantroline)

Hamujący wpływ 1,10-phenantroliny na syntezę RNA w wyizolowanych jądrach komórkowych z grasicy cielęcia. Jądra (2 x 10^7 jąder/ml) inkubowano w obecności wzrastających stężeń 1,10-phenantroliny (0,5-10 mM) 1 h w 37°C. Następnie 100 µl próbki zawiesiny jąder dodawano do 250 µl standardowej mieszaniny inkubacyjnej, prowadzono inkubację w 25°C przez 30 min. Ilość włączonego prekursora H3-UTP określano po osadzeniu jąder na sączkach z włókna szklanego Whatman. (Kontrola: 100% syntezy RNA w jądrach bez inkubacji z 1,10-phenantroliną)

ses transcriptional process in nuclei was inhibited by this chelating agent of zinc ions, in a concentration dependent manner (Fig. 5).

Next we examined the influence of exogenous zinc ions on RNA synthesis in isolated calf tyhmus nuclei. In this case, distinct decrease in transcriptional activity of this system was observed as well (Fig. 6). The magnitude of inhibiting effect of zinc ions was dependent on the zinc/nuclei concentration ratio. The increasing amount of exogenous zinc ions resulted in the increase of inhibitory effect of this metal. When the calf thymus nuclei

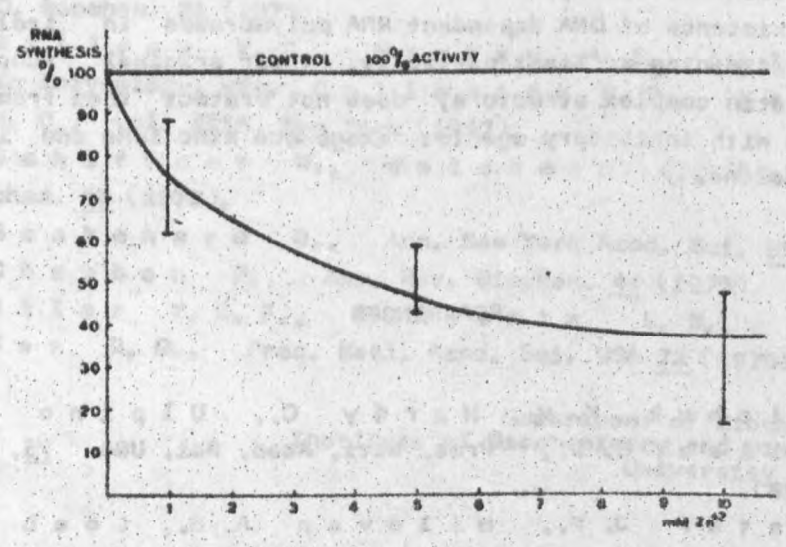


Fig. 6. Effect of various concentrations of zinc ions on yield of transcription in isolated calf thymus nuclei. Nuclei (10^7 nuclei/ml) were incubated in presence of increasing concentrations of zinc ions (1-10 mM) 1 h at 37°C. After that, 100 µl samples of nuclei suspension were added to 250 µl standard reaction mixture and incubation was performed at 25°C for 30 min. TCA precipitable counts were determined as in Fig. 5 (Control: 100% RNA synthesis in nuclei without incubation with zinc ions)

Wpływ różnych stężeń jonów Zn^{2+} na poziom transkrypcji w jądrach komórkowych izolowanych z grasicy cielęcia. Jądra (10^7 jąder/ml) inkubowano w obecności wzrastających stężeń jonów Zn^{2+} (1-10 mM) przez 1 h w 37°C. Następnie 100 µl próbki zawiesiny jąder dodawano do 250 µl standardowej mieszaniny inkubacyjnej, prowadzono inkubację w 25°C przez 30 min. Ilość włączanego prekursora H3-UTP określano jak na rys. 5. (Kontrola: 100% syntezy RNA w jądrach komórkowych bez inkubacji z jonami Zn^{2+})

were incubated with equivalent concentrations of both 1.10-phenantroline and exogenous zinc ions no inhibitory effect was observed. RNA synthesis in these nuclei was continued without disturbances.

Experiments concerning influence of exogenous zinc ions on RNA synthesis in isolated, functionally active calf thymus nuclei confirmed our earlier results obtained for purified RNA polymerases. Inhibiting effect of 1.10-phenantroline on RNA synthesis in nuclei demonstrated indirectly the existence of intrinsic zinc in protein of these enzymes, which is important for their transcriptional activity.

The existence of DNA dependent RNA polymerases in isolated nuclei, retaining at least partially, their original function and chromatin complex structure, does not protect them from interaction with inhibitory agents: exogenous zinc ions and 1.10-phenantroline.

REFERENCES

[1] Falchuk K. H., Hardy C., Ulpino L., Vallee B. L., Proc. Natl. Acad. Sci. USA 75, 9 (1978).
[2] Slater J. P., Mildvan A. S., Loeb L.A., BBRC 44 (1971).
[3] Valenzuela P., Morris R., Farse A., Levinson W., Rutter W. J., BBRC 53, (1973).
[4] Poiesz B. J., Battula N., Loeb L. A., BBRC 56, (1974).
[5] Coleman J. E., BBRC 60 (1974).
[6] Williams K. R., Schofield P., BBRC 64 (1975).
[7] Auld D. S., Atsuya J., Campino C., Valenzuela P., BBRC 69 (1976).
[8] Lattke H., Weser U., FEBS Lett. 65 (1976).
[9] Falchuk K. H., Mazus B., Ulpino L., Vallee B. L., Biochemistry 15 (1976).

[10] Falchuk K. H., Ulpino L., Mazue B., Vallee B. L., BBRC 74 (1977).
[11] Rose K. M., Allen M. S., Crawford I. L., Jacob S. T., Eur. J. Biochem. 68 (1978).
[12] Mildvan A. S., Ann Rev. Biochem. 43 (1974).
[13] Mildvan A. S., Loeb A. L., CRC Critical Reviewe in Biochemistry (1979).
[14] Brudzyńska K., Walter Z., Czajkowska A., Zesz. Nauk. UŁ II, 23 (1979).
[15] Schaller H., Nuselein C., Bonhoffer F. J., Kurz C., Nietzmann I., Eur. J. Biochem. 26 (1972).
[16] Allfrey V. G., Mirsky A. E., [in:] Methode in Enzymology, eds. Collowick S. P., Kaplan N. O., vol. XIIA, New York (1967).
[17] Schaffner W., Weissman C., Anal. Biochem. 56 (1973).
[18] Scatchard G., Ann. New York Acad. Sci. 51 (1948).
[19] Chambon P., Ann. Rev. Biochem. 44 (1975).
[20] Sklar V. E. F., Schwartz L. B., Roeder R. G., Proc. Natl. Acad. Sci. USA 72 (1975).

Department of Biochemistry
Institute of Biochemistry and Biophysics
University of Lodz

A. Kubiak, K. Brudzyńska, Z. Walter

WPŁYW JONÓW CYNKU NA TRANSKRYPCJĘ U EUKARIONTÓW

Oczyszczone polimerazy RNA A, B i C zależne od DNA izolowane z grasicy cielęcej zawierają znaczne ilości cynku. Metodą atomowej spektrometrii absorbcyjnej wykazano obecność 6,7; 5,35 i 2,6-4,1 g-atomów cynku, odpowiednio na mol polimerazy A, B i C. Enzymy te były hamowane w obecności różnych stężeń (10^{-5}-10^{-4} M) 1,10-phenantroliny. Jednakże dodanie jonów cynku nie odwracało w pełni aktywności enzymów inkubowanych uprzednio z 1,10-phenantrolinę. Egzogenne jony cynku redukują całkowitę syntezę RNA katalizowaną przez polimerazy RNA z grasicy cielęcej. Prócz miejsc, które wiążą cynk w sposób specyficzny i stechiometryczny, enzymy te posiadają inne klasy miejsc wiązania z wysokim i niskim

powinowactwem. Związanie egzogennego cynku w tych dodatkowych miejscach wiązania na powierzchni enzymu hamuje aktywność polimeraz. Ponadto, zbadano tempo syntezy RNA w jądrach komórkowych wyizolowanych z grasicy cielęcej, w obecności: 1,10-phenantroliny i jonów cynku. Podobnie jak w przypadku oczyszczonych polimeraz RNA proces transkrypcji w jądrach był przez oba te związki hamowany.